between SRB generated sulfide and its subsequent oxidation via oxygen consumption back to sulfate, will perpetuate the risk. Results here identify that biogeochemical linkages of potential interventions for management and reclamation strategies must be considered to avoid deleterious impacts.

Theory / Method / Workflow

Over 150 sampling campaigns were carried out on BML from June 2015 to August 2019. These sampling campaigns included a physciochemical profile of the BML water cap (temperature, pH, oxygen, specific conductivity, oxidation reduction potential (ORP) YSI Professional Plus 6-Series Sonde;) and collection of a minimum of three depth-dependent set of water samples for both geochemical and microbiological characterization. All methods and sampling protocols have been described elsewhere (Risacher et al. 2018, Arriaga et al. 2019 and Mori et al. 2019). Briefly, all sampling equipment and sample containers were prepared by soaking in 5% (v/v) HCl for 12 h followed by seven rinses with ultrapure water (18.2Ωm cm-1, MilliQ, Millipore) (inorganic analytes). Bulk water was collected in a 9 L Van Dorn bottle (Wilco Model: E-411-19XX-H62) and samples were collected for measurement of CH_4, bulk carbon (DOC and POC), and dissolved (< 0.45 µm) aqueous species of Fe^{2+}/ Fe^{3+}, NH_4^+, NO_2^-, NO_3^-, ΣH_2S, and SO_4^{2-} (as described in detail in Risacher *et al*. 2018, Arriaga *et al*. 2019). In addition, samples were collected for cultivation independent analyses of water cap microbial communities. Briefly, microbial cells were collected by filtering ~ 1.5L of water from each sampling depth through 0.22 um Rapid-Flow sterile disposable filters (Thermo Fisher Scientific), stored at -20 C until DNA extraction followed by microbial 16S rRNA gene amplicon sequencing and, for select samples, metagenomics (i.e. functional gene) analyses (shotgun sequencing) using Illumina adapted primers and following standard protocols of the Earth Microbiome Project (Mori et al. 2019).

Results, Observations, Conclusions

Samples collected during the thermally stratified summer seasons for 2015 and 2016 decreasing oxygen concentrations with depth, ranging from 70-90% saturation in the upper epilimnetic region to < 10 uM or 0.25 mg/L at the FFT water interface (Risacher et al. 2018, Arriaga et al. 2019). Further results from these two years identified that dissolved aqueous methane (2015) and dissolved aqueous methane and ammonia (2016) were the key OCC negatively impacting water cap oxygen concentrations observed in field geochemical data (Risacher et al. 2016) as well as in oxygen consumption experiments (Arriaga et al 2019). The alum amendment in the fall of 2016 was followed by observed higher oxygen concentrations within the epilimnion during the summer of 2017 (Figure 1). However, while there was some increase in the oxygen concentration in the upper epilimnetic waters (~up to concentrations of 9 mg/L or ~ 280 uM), evidence of anoxia in BML bottom waters observed for the first time in August 2017 was also observed again in 2018 (Figure 2) indicating an increase in overall oxygen consumption.

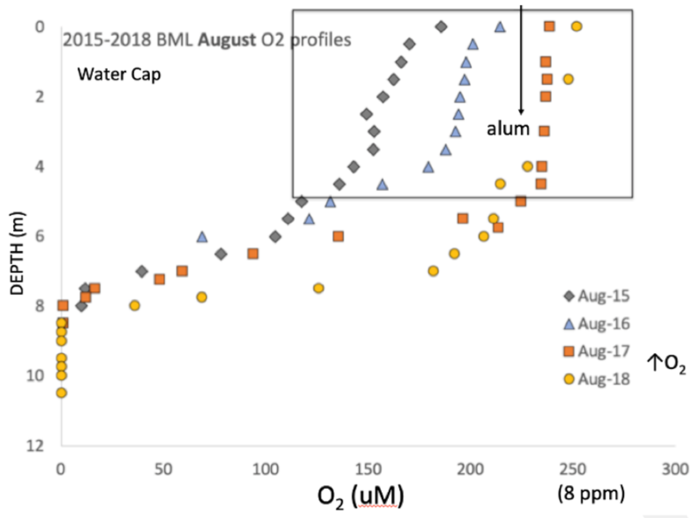

Figure 1. BML August depth dependent water cap oxygen concentration profiles (2015-2018). The increase in epilimnetic oxygen concentrations observed in 2017-2018 post alum addition (fall 2016) are highlighted in the box.

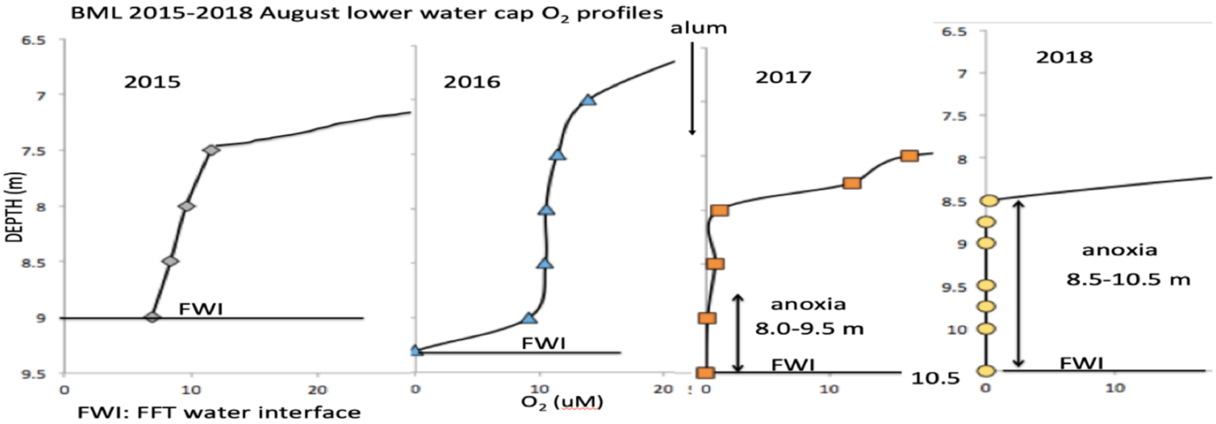

Figure 2. BML August hypolimnetic water cap oxygen profiles for 2015-2018. The first observed anoxia within the lower BML water cap occurred during August 2017, the first summer post alum addition and was repeated in 2018.

The occurrence of anoxic conditions can be explained by the (unanticipated) consequences of stimulating algal production within BML. The alum addition to BML was trialed to increase clarity, enable greater primary productivity and thus boost oxygen concentrations in the upper waters. Consistent with this hypothesized outcome, the upper waters of BML in the summer of 2017 showed the highest oxygen concentrations observed since 2015 as already described above (Figure 1). However, proxies consistent with increased algal biomass are also evident (Figure 3). Increased concentrations of photosynthetic pigment (Figure 3a; i.e. algal biomass) would, in turn, support greater invertebrate and periphyton biomass (Figure 3a-c) as well as greater decomposition by heterotrophic microbial activity (Figure 3d).

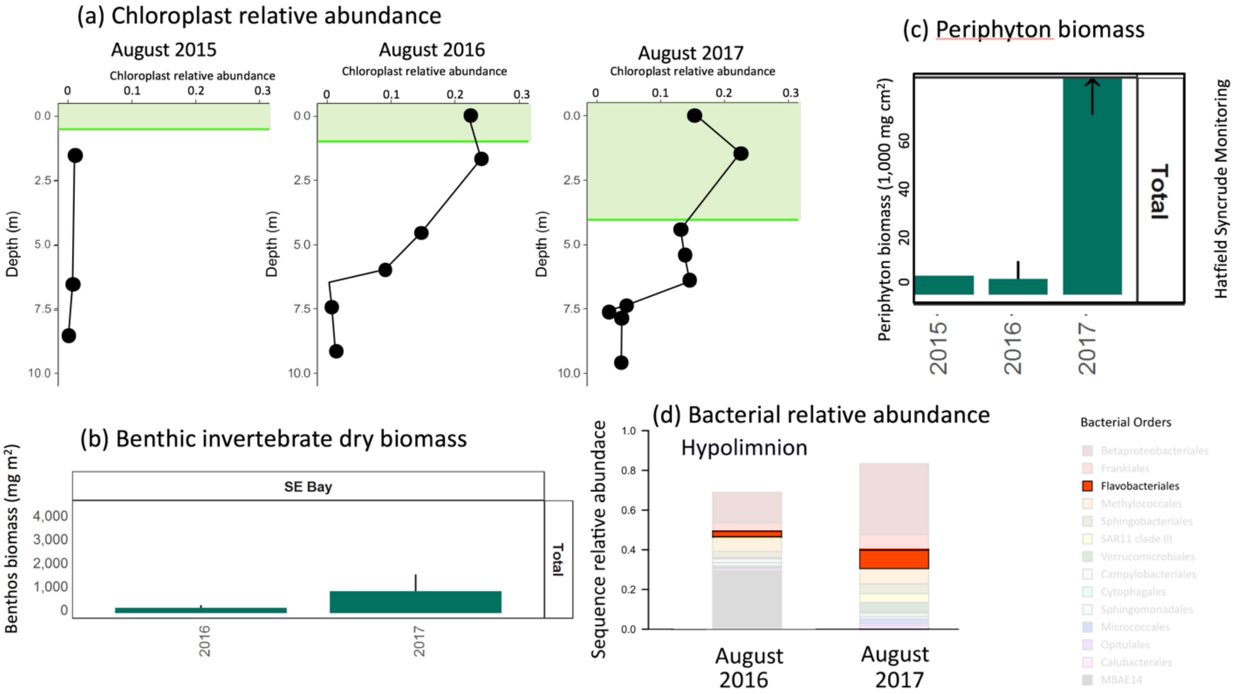

Figure 3. Proxies of labile organic carbon for pre and post alum addition in BML: (a) relative abundance of Chla; (b) biomass of benthic invertebrates; (c) periphyton biomass and (d) relative abundance of aerobic heterotrophic bacterial order Flavobacteriales (chloroplast abundance, benthic invertebrate and periphyton biomass values provided by Hatfield Consulting as part of the Syncrude Canada BML monitoring program).

Under ice oxygen concentrations observed for the BML water cap over 2017 – 2019 also support this shift in ecology of BML (Figure 4). These profiles indicate a decreasing trend in oxygen concentrations throughout the water cap consistent with greater consumption through heterotrophic respiration (Figure 4).

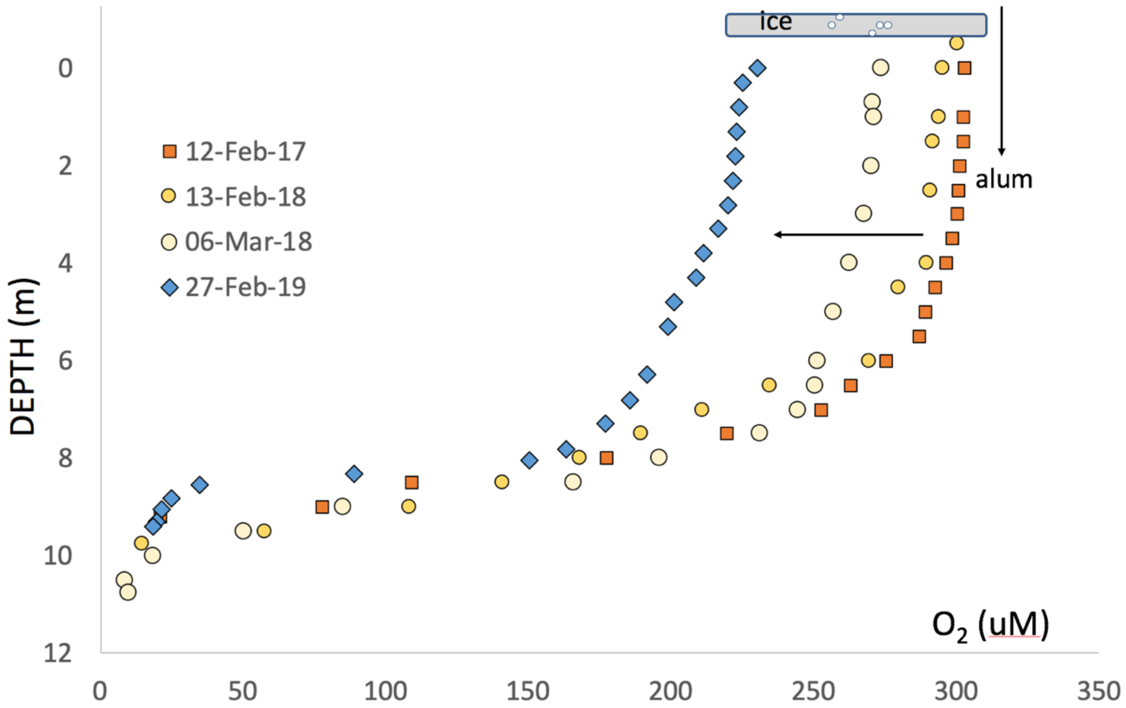

Figure 4. BML water cap under ice oxygen profiles from February 2017 (first winter pos alum addition) through February 2019 showing a decrease in epilimnetic oxygen concentrations consistent with greater respiration and heterotrophic carbon degradation.

Anoxia is of concern, as it enables sulfide generation by sulfate reducing bacteria (SRB) directly within the water cap (eqn 1). At the alkaline pH values of BML, sulfide oxidizes rapidly in the presence of oxygen (eqn 2), through both abiotic and microbial (sulfur oxidizing bacteria, SOB) catalysis and thus the concentrations of available organic carbon and sulfate will be key drivers of associated oxygen consumption.

Sulfate reduction by SRB

$$SO_4^{2-} + 2CH_2O \rightarrow HS^- + 2HCO_3^- + H^+ \qquad \text{(Eqn 1)}$$

abiotic and/or sulfur oxidizing bacteria (SOB) catalysis

$$HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+ \qquad \text{(Eqn 2)}$$

BML water cap sulfate concentrations are ~ 10x those of maximum observed summer surface (i.e. 0-4m) associated oxygen concentrations (i.e. 250-300 uM; 7.8 – 9.4 mg/L) and 250X those observed in the deeper water oxygen concentrations (i.e. > 6.5 m; <10 uM; 0.32 mg/L) identifying substantive capacity to deplete BML oxygen concentrations. Further, the rapid recycling between SRB generated sulfide and its subsequent oxidation via oxygen consumption back to sulfate, will perpetuate the risk to the maintenance of an oxic zone within BML. Thus, sulfur cycling within BML poses a significant risk to the stability of a persistent oxygenated zone.

Novel/Additive Information

Here, results identify that an alum amendment added to improve clarity and stimulate greater primary productivity that would boost oxygen concentrations precipitated a cascade of interlinked biogeochemical processes that have lead to the emergence of a new significant risk to BML water cap oxygen concentrations through water cap sulfur cycling. Results identified that sulfur cycling and specifically the potential for generation of sulfide within the water cap is an important emerging risk to oxygen levels within the BML water cap. The generation of labile organic carbon from the increased algal biomass in the upper waters post alum addition, increased oxygen consumption within the bottom BML water cap, precipiting anoxic conditions during late summer; anoxia had not previously been observed prior to alum addition, during the summer stratification period in BML. This newly established anoxia enabled anaerobic sulfur cycling directly within the water cap, and the emergence of water cap associated sulfide. The substantively higher sulfate concentrations relative to oxygen concentrations within the BML water cap, and the rapid regeneration of sulfate through oxygen driven sulfide oxidation that can perpetuate this oxygen stripping mechanism, collectively underscore the significance of this emergent risk to BML oxygen concentrations and the need to more fully constrain the biogeochemistry of mining waste contexts in management and reclamation.

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